

### AMENDMENTS TO THE CLAIMS

1. (Currently amended) A process for recovering polyhydroxyalkanoates (PHAs) from cellular biomass of bacteria, said biomass being obtained by fermentation and in the form of a cellular biomass slurry in aqueous suspension and with a dry cellular content not inferior to about 18% by weight, characterized in that it comprises the steps of: i) submitting the concentrated cellular biomass slurry to concomitant operations of injection of PHA solvent, of ~~vigorous~~ agitation which maintains solvent and PHA cellular biomass in suspension and in contact, and of ~~quick~~ heating in the interior of a reactor, in order to provoke the rupture of the walls of the cellular biomass and the dissolution of the PHA contained in the latter, wherein the steps of heating the fermented cellular biomass, of rupturing the cell walls of said cellular biomass, and of dissolving the PHA contained in the latter are carried out in a total time that is sufficiently short to allow obtaining a PHA with a molecular weight at minimum of about 850,000 Da, from a biomass containing PHA with a molecular weight at minimum of about 1,000,000 Da, and to form a suspension comprising PHA solvent enriched with dissolved PHA, water remaining from the cellular biomass slurry and insoluble residues of the concentrated cellular biomass; ii) submitting the suspension formed in the reactor to a separation step, for recovering the solvent, enriched with the dissolved PHA, from the insoluble residues of the remaining cellular biomass; iii) ~~rapidly~~ cooling by expansion, through heat exchange with another cooler stream and/or by cooling by means of heat exchangers the PHA solvent solution enriched with PHA to a temperature which is sufficient to substantially precipitate all the dissolved PHA; iv) cold micro-filtrating at 45°C or less the PHA suspension precipitated in the PHA solvent containing water and impurities dissolved therein, in order to separate a concentrated paste of precipitated PHA; v) submitting the paste concentrated with PHA to simultaneous operations of washing with water, heating and agitation, in order to promote the evaporation of a certain amount of solvent which is adequate to obtain a suspension containing PHA granules of high porosity and which are brittle and easily shearable, the remaining solvent, and water; vi) submitting the washed and heated PHA granules to agitation and shearing, so as to rapidly break them, while processing the extraction of the residual solvent by injecting water vapor into the suspension containing the remaining solvent and water, in order to obtain ~~purified~~ PHA particles

Application No. 10/596,077  
Amendment dated March 8, 2010  
Reply to Office Action of October 7, 2009

Docket No.: 06290/0204400-US0

with a purity of 99% or greater in the suspension, wherein the recovery rate of PHA particles is 90% or greater; and vii) separating the purified PHA particles from the suspension.

2. (Original) The process as set forth in claim 1, characterized in that the PHA solvent used is selected from the group of solvents consisting of: butyl acetate, isobutyl acetate, amyl acetate, isoamyl acetate, isobutyl alcohol, 1-butanol, 1-pentanol (amyl alcohol), 2-methyl-1-butanol, 3-methyl-1-butanol, (isoamyl alcohol), 3-pentanol, 1-hexanol, cyclohexanol, propyl propionate, butyl propionate, isobutyl propionate, ethyl butyrate, isobutyl isobutyrate, and mixtures of these solvents.
3. (Original) The process as set forth in claim 2, characterized in that the solvent used is the isoamyl alcohol, or isomeric mixtures of isoamyl alcohol.
4. (Original) The process as set forth in claim 3, characterized in that the isoamyl alcohol is obtained by fractionizing the fusel oil as by product of the ethanol fermentation, the fusel oil being primordially composed by isoamyl alcohol and isomers thereof, besides impurities, such as: ethanol, n-propanol, isobutanol, n-butanol, and water.
5. (Original) The process as set forth in claim 4, characterized in that the PHA is selected from the group consisting of poly-3-hydroxybutyrate (PHB), poly (hydroxybutyrate-co-hydroxyvalerate) PHBV, and mixtures of these polymers and copolymers.
6. (Original) The process as set forth in claim 5, characterized in that the PHA is produced by bacterial fermentation, using microorganisms which are able to biosynthesize PHA using, as main raw material, sugars extracted from the sugarcane, and in that the main energetic source used to generate the thermal energy and the electric energy required by the process is the sugarcane bagasse.
7. (Original) The process as set forth in claim 1, characterized in that the PHA is selected from the group consisting of poly-3-hydroxybutyrate (PHB), poly (hydroxybutyrate-co-hydroxyvalerate) PHBV, and mixtures of these polymers and copolymers.

Application No. 10/596,077  
Amendment dated March 8, 2010  
Reply to Office Action of October 7, 2009

Docket No.: 06290/0204400-US0

8. (Original) The process as set forth in claim 1, characterized in that the bacterial cellular biomass obtained through fermentation and to be processed is previously thermally inactivated.

9. (Currently amended) The process as set forth in claim 1, characterized in that the step of injecting solvent into the concentrated cellular biomass slurry comprises operations of injecting liquid PHA solvent and PHA solvent in the form of vapor, in order to provoke the heating of the cellular biomass to a temperature between about ~~90~~90°C and the boiling temperature of the solvent at a substantially atmospheric pressure, and to form: a liquid phase comprising PHA solvent enriched with PHA and water remaining from the cellular biomass slurry; a solid phase defined by the insoluble residues of the residual cellular biomass; and a vapor phase containing vapors of water and of the PHA solvent.

10. (Original) The process as set forth in claim 9, characterized in that it comprises the additional step of extracting the vapor phase from the interior of the reactor.

11. (Original) The process as set forth in claim 10, characterized in that the PHA paste is washed with a water stream coming from the condensation of the vapor phase extracted from the reactor during the step of cellular rupture and PHA dissolution.

12. (Original) The process as set forth in claim 1, characterized in that the concentrated cellular biomass slurry is obtained by submitting the cellular biomass in suspension in the fermented culture medium to operations of flocculation and concentration of the cellular biomass.

13. (Currently amended) The process as set forth in claim 12, characterized in that the cellular biomass in suspension in the fermented culture medium to be supplied to the process is further diluted in water, in order to present a fermented material: water mass ratio up to about ~~3,0~~1,0:1,0.

14. (Original) The process as set forth in claim 13, characterized in that the flocculation operation comprises a step of coagulating the cellular biomass effected by acidification of the diluted cellular biomass to a pH from about 1.5 to about 5.5 and through the addition of an

alkalizing agent, until reaching a pH from 7 to about 12, the flocculation operation of the cellular biomass containing accumulated PHA being carried out through the addition of a flocculating agent.

15. (Original) The process as set forth in claim 14, characterized in that the acidification of the diluted cellular biomass is obtained by adding an acid defined by at least one of the sulfuric and phosphoric acids.

16. (Original) The process as set forth in claim 14, characterized in that the alkalizing agent comprises calcium hydroxide.

17. (Original) The process as set forth in claim 14, characterized in that the acidification is carried out in order to obtain a pH from about 2.0 to about 3.0, the addition of the alkalizing agent being made so as to adjust the pH of the suspension of the diluted cellular biomass to a range between about 7 and about 12.

18. (Original) The process as set forth in claim 13, characterized in that the flocculation operation comprises a step of coagulating the cellular biomass by adding an alkalizing agent until reaching a pH from about 7 to about 12, the flocculation of the cellular biomass containing accumulated PHA being achieved through the addition of a flocculating agent.

19. (Original) The process as set forth in claim 18, characterized in that the alkalizing agent comprises calcium hydroxide.

20. (Original) The process as set forth in claim 12, characterized in that the flocculation operation comprises a step of coagulating the cellular biomass by acidifying the cellular biomass to a pH from about 1.5 to about 5.5 and by adding an alkalizing agent until reaching a pH from about 7 to about 12, the flocculation of the cellular biomass containing accumulated PHA being achieved through the addition of a flocculating agent.

Application No. 10/596,077  
Amendment dated March 8, 2010  
Reply to Office Action of October 7, 2009

Docket No.: 06290/0204400-US0

21. (Original) The process as set forth in claim 20, characterized in that the acidification of the diluted cellular biomass is obtained by adding an acid defined by at least one of the sulfuric and phosphoric acids.

22. (Original) The process as set forth in claim 20, characterized in that the alkalizing agent comprises calcium hydroxide.

23. (Original) The process as set forth in claim 20, characterized in that the acidification is carried out in order to obtain a pH from about 2.0 to about 3.0, the addition of the alkalizing agent being made so as to adjust the pH of the suspension of the diluted cellular biomass to a range between about 7 and about 12.

24. (Original) The process as set forth in claim 12, characterized in that the flocculation operation comprises a step of coagulating the cellular biomass by adding an alkalizing agent until reaching a pH from about 7 to about 12, the flocculation of the cellular biomass containing accumulated PHA being achieved through the addition of a flocculating agent.

25. (Original) The process as set forth in claim 24, characterized in that the alkalizing agent comprises calcium hydroxide.

26. (Original) The process as set forth in claim 12, characterized in that the concentration of the flocculated biomass cells is achieved by at least one of the operations of decantation and centrifugation.

27. (Original) The process as set forth in claim 12, characterized in that the cellular biomass slurry in suspension in the fermented flocculated culture medium is subjected to washing with water and to a concentration to the range of 18%-45%, preferably of 25%-45% by weight of dry cellular biomass.

Application No. 10/596,077  
Amendment dated March 8, 2010  
Reply to Office Action of October 7, 2009

Docket No.: 06290/0204400-USO

28. (Original) The process as set forth in claim 27, characterized in that the step of washing and concentrating the cellular biomass slurry is achieved by simultaneously submitting the latter to a flow of water and to the effects of centrifugal force.
29. (Original) The process as set forth in claim 1, characterized in that the liquid PHA solvent which is injected into the cellular biomass slurry is heated.
30. (Original) The process as set forth in claim 1, characterized in that the step of separating the PHA solvent enriched with PHA dissolved therein from the insoluble residues of the remaining biomass that are contained in the suspension formed inside the reactor comprises at least one of the operations of membrane micro-filtration and of filtration in precoat filters.
31. (Original) The process as set forth in claim 12, characterized in that the step of separating the PHA solvent enriched with PHA dissolved therein from the insoluble residues of the remaining biomass that are contained in the suspension formed inside the reactor comprises a step of subjecting said suspension to a separation by the effect of centrifugal force of low intensity.
32. (Original) The process as set forth in claim 31, characterized in that the centrifugal force of low intensity, which is used in the step of separating, from the PHA solution enriched with PHA dissolved therein, the insoluble residues of the remaining biomass which are contained in the suspension formed inside the reactor, is obtained by means of hydrocyclones, producing a suspension with low concentration of said residues and another suspension concentrated with said residues.
33. (Original) The process as set forth in claim 32, characterized in that the suspension of low concentration of biomass insoluble residues which leaves the hydrocyclones is rapidly submitted to an additional separation step for completely removing the residues before being submitted to the cooling step.
34. (Original) The process as set forth in claim 33, characterized in that the additional separation step is effected by membrane micro-filtration, in order to produce a solution of PHA dissolved in

the PHA solvent, free of insoluble residues, and a suspension concentrated in biomass insoluble residues and containing a fraction of PHA dissolved in the PHA solvent, water, ashes, and color compounds dissolved in the PHA solvent.

35. (Original) The process as set forth in claim 34, characterized in that the suspension concentrated in insoluble residues of cellular biomass is subjected to a filtration step, in order to produce a meal containing the biomass insoluble residues and a filtrated solution of PHA dissolved in the solvent, free of insoluble residues and which will be rapidly submitted to the cooling step.

36. (Original) The process as set forth in claim 34, characterized in that the solution of PHA dissolved in the PHA solvent and free of insoluble residues represents about 60-90% by weight of the suspension in micro-filtration, the suspension concentrated in residues of cellular biomass representing about 10-50% by weight of said suspension in micro-filtration.

37. (Original) The process as set forth in claim 32, characterized in that the suspension concentrated with biomass insoluble residues which leaves the hydrocyclones is submitted to a filtration step for separating the biomass insoluble residues before being submitted to the cooling step.

38. (Currently amended) The process as set forth in claim 1, characterized in that the step of cold micro-filtrating the suspension of PHA precipitated in the PHA solvent is carried out in order to produce a PHA paste with a concentration of PHA from about ~~3.5% to 8.0%~~ 3.5% to 8.0% w/w.

39. (Original) The process as set forth in claim 1, characterized in that it further comprises the final step of drying the PHA particles separated from the aqueous medium from which the solvent was depleted.

40. (Original) The process as set forth in claim 1, characterized in that the water and PHA solvent vapors, which are generated in the several stages of the process, are condensed and separated in two liquid phases: one solvent-rich liquid phase which returns to the process in the

Application No. 10/596,077  
Amendment dated March 8, 2010  
Reply to Office Action of October 7, 2009

Docket No.: 06290/0204400-USO

PHA extraction and recovery step; and another solvent-poor liquid phase, which is recirculated in the process to allow recovering the PHA solvent contained therein.

41. (Canceled)

42. (Canceled)

43. (Currently amended) The process as set forth in claim 1, characterized in that the PHA granules obtained in step (vi), after drying, have a particle average size in the range from 40 to 400 ~~pm~~ ppm and preferably in the range from 100 to 200 ~~pm~~ ppm.

44. (Currently amended) A poly-3-hydroxybutyrate (PHB) with a purity of 99% or greater, and having a molecular weight at minimum of about 850,000 Da obtained from a biomass containing PHB with a molecular weight at minimum of about 1,000,000 Da, characterized in that it is produced according to claim 1 by a process for recovering polyhydroxyalkanoates (PHAs) from cellular biomass of bacteria, said biomass being obtained by fermentation and in the form of a cellular biomass slurry in aqueous suspension and with a dry cellular content not inferior to about 18% by weight, characterized in that it comprises the steps of: i) submitting the concentrated cellular biomass slurry to concomitant operations of injection of PHA solvent, of agitation which maintains solvent and PHA cellular biomass in suspension and in contact, and of heating in the interior of a reactor, in order to provoke the rupture of the walls of the cellular biomass and the dissolution of the PHA contained in the latter, wherein the steps of heating the fermented cellular biomass, of rupturing the cell walls of said cellular biomass, and of dissolving the PHA contained in the latter are carried out in a total time that is sufficiently short to allow obtaining a PHA with the molecular weight at minimum of about 850,000 Da, from the biomass containing PHA with the molecular weight at minimum of about 1,000,000 Da, and to form a suspension comprising PHA solvent enriched with dissolved PHA, water remaining from the cellular biomass slurry and insoluble residues of the concentrated cellular biomass; ii) submitting the suspension formed in the reactor to a separation step, for recovering the solvent, enriched with the dissolved PHA, from the insoluble residues of the remaining cellular biomass; iii) cooling by expansion, through heat



Application No. 10/596,077  
Amendment dated March 8, 2010  
Reply to Office Action of October 7, 2009

Docket No.: 06290/0204400-US0

exchange with another cooler stream and/or by cooling by means of heat exchangers the PHA solvent solution enriched with PHA to a temperature which is sufficient to substantially precipitate all the dissolved PHA; iv) cold micro-filtrating at 45°C or less the PHA suspension precipitated in the PHA solvent containing water and impurities dissolved therein, in order to separate a concentrated paste of precipitated PHA; v) submitting the paste concentrated with PHA to simultaneous operations of washing with water, heating and agitation, in order to promote the evaporation of a certain amount of solvent which is adequate to obtain a suspension containing PHA granules of high porosity and which are brittle and easily shearable, the remaining solvent, and water; vi) submitting the washed and heated PHA granules to agitation and shearing, so as to rapidly break them, while processing the extraction of the residual solvent by injecting water vapor into the suspension containing the remaining solvent and water, in order to obtain PHA particles in the suspension, wherein the recovery rate of PHA particles is 90% or greater; and vii) separating the purified PHA particles from the suspension.